JNPDD
Journal of Natural Product for Degenerative Diseases
https://journal.univpancasila.ac.id/index.php/Natural

Preparation, Evaluation, and *In Vitro* Antiacne Activity of *Cymbopogon citratus* DC and *Cymbopogon nardus* (L.) Rendle Essential Oil Microemulsion

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Received: 25 August 2023 / Accepted: 7 September 2023

ABSTRACT: This study was conducted to formulate and evaluate a topical anti-acne microemulsion containing essential oils of *Cymbopogon citratus* DC and *Cymbopogon nardus* (L). Using the disc diffusion method, the antibacterial activity of the essential oils of *C. citratus* and *C. nardus* against *Propionibacterium acne* and *Staphylococcus epidermidis* was investigated. After identifying the active concentration, the formula for a topical microemulsion was devised and tested for physical and chemical parameters. The results revealed that the essential oils of *C. citratus* and *C. nardus* had antimicrobial activity against *P. acnes* and S. *epidermidis*. The results demonstrated that the obtained microemulsion meets the physical and chemical requirements and possesses antimicrobial activity. It can be concluded that the essential oils extracted from *C. citratus* and *C. nardus* have the potential for further development and commercial application in the treatment of acne.

KEYWORDS: *Cymbopogon citratus; Cymbopogon nardus; microemulsion; Propionibacterium acne; Staphylococcus epidermidis*

1. INTRODUCTION

Acne vulgaris, often known as acne, commonly manifests as pimples and mostly affects the pilosebaceous follicle. It primarily denotes inflammation-causing open and closed comedones. papules, pustules, and nodules are a few different conditions [1]. The principal microbes that cause the disease include *Propionibacterium acnes* and *Staphylococcus epidermidis* [2]. These microorganisms multiply quickly, which ultimately lead to the appearance of acne.

Numerous pharmaceuticals, including antibiotics and chemotherapeutic medicines of both synthetic and natural origin, are on the market to address the A. vulgaris problem. both internally and externally to treat the condition [3]. For A. vulgaris has a milder disease, and only topical medication is useful. nonetheless, systemic therapy coupled with applying topically is a preferred method. herbal treatments are expanding adoption globally over the ones that are now available as the bulk of the current formulas have negative side effects include dry skin, skin eruption, erythema, wrinkles, pruritus, and development of opposition

According to a previous study [5], it has been observed that both the crude extract and extracted essential oil of *Cymbopogon citratus* DC and *Cymbopogon nardus* (L) Rendle demonstrate antibacterial characteristics. Consequently, the scope of the investigation has been broadened to evaluate the potential of the essential oil in combating acne by targeting *P. acnes* and *S. epidermidis*. Additionally, microemulsion formulations were created. Microemulsions offer numerous advantages in comparison to conventional formulations, including improved drug solubility, favorable thermodynamic stability, simplified manufacturing processes, and better penetration for topical application [6,7]. The enhanced ability to dissolve substances and the reduced size of droplets contribute to improved affinity for biological membranes, facilitating the regulated transportation of drugs. This treatment is highly beneficial for addressing acne due to its ability to stimulate skin attachment and subsequently increase the concentration of antibacterial agents in the targeted region [8].

How to cite this article: Datubara ME, Kumala S, Rahmat D. Preparation, evaluation, and in vitro antiacne activity of *Cymbopogon citratus* DC and *Cymbopogon nardus* (L.) Rendle essential oil microemulsion. JNPDD. 2023; 1(1): 31-40.

https://doi.org/10.58511/jnpdd.v1i1.5463
ISSN: 0000-0000

2. MATERIALS AND METHODS

2.1. Materials

The materials in this study were *Cymbopogon nardus* (L.) Rendle essential oil, *Cymbopogon citratus* DC essential oil, chloramphenicol 30mcg/disc, Tween 80 (E Merck), Glyceryl caprylate (Dermasoft GMY Skin Dewi, PEG 40 Hydrogenated Castor oil (Ciptakimia), Capric triglyceride (Kimia Raya), BHIA media (Micro-Boc), nutrient agar media (Micro-Boc), *Propionibacterium acnes* culture (Pa121), *Staphylococcus epidermidis* culture (Se151) (University of Indonesia microbiology laboratory).

2.2. Distillation of essential oils

The oils utilized in this investigation were acquired by the process of steam distillation of the fresh leaves of *C. citratus* and *C. nardus*. The leaves selected for this purpose were of 6-9 months in age and measured between 25-75 cm in length. *C. citratus* and *C. nardus* essential oil quality parameters. The yield was calculated by comparing the amount of extract obtained with the amount of initial simplisia used.

2.3 Determination of *C. citratus* and *C. nardus* oil quality parameters

Determination of quality requirements for *C. citratus* essential oil refers to ISO 3217:1974(en) and *C. nardus* essential oil refer to ISO 3849:2003(E). The parameters of color testing were carried out organoleptically. The density test was carried out using a pycnometer. The refractive index test was carried out using a refractometer. The solubility in ethanol testing was carried out using the synthesis method [9].

2.4. Chemical composition of *C. citratus* and *C. nardus* essential oil compounds with GC-MS

Gas Chromatography-Mass Spectroscopy (GC-MS) was employed to identify the components of the produced essential oil. The KG-SM apparatus employed in this study consisted of a Shimadzu QP 2010S gas chromatograph equipped with a Rastek RXi-5MS column. The column had a length of 30 meters, a diameter of 0.25 millimeters, and a thickness of 0.25 micrometers The essential oil was injected with a volume of 1 µL in a split ratio of 1:25 and a solvent delay of 2 minutes with methanol as a solvent. The temperature of the column was initially set to 100°C and held for a duration of 5 minutes. Subsequently, the temperature was increased at a rate of 10°C per minute until it reached a final temperature of 290°C, which was maintained for a period of 30 minutes. The temperatures of the injector and ion source were set at 250°C and 290°C, respectively, for electron ionization (EI) at 70 eV. The carrier gas employed in this study was Helium (He), with a flow rate of 0.5 ml/min and a velocity ratio of 1:50. The scan range of the SM (single mass) analysis method spans from m/z 28 to 600. The identification of the compound's structure was conducted by comparing the fragmentation pattern of the molecule with a recognized standard in the library database. Every each peak observed in the chromatogram exhibits a distinct retention time. Zone of inhibition of *C. citratus* and *C. nardus* essential oil.

2.6. Antimicrobial activity measurement

2.6.1. Inhibition diameter test

Sterilized liquid nutrient agar was put into each petri dish as much as 20 ml and allowed to solidify at room temperature. Nutrient agar medium was used for *Staphylococcus epidermidis* bacteria and blood agar medium was used for *Propionibacterium acnes* bacteria. A sterile cotton swab was dipped into a suspension of test bacteria that had the same turbidity as the Mc Farland 0.5 standard and then pressed against the tube wall until the cotton swab was not too wet, then applied to the agar medium until it was flat, then a sterile disc paper with a diameter of 6 mm was saturated in the preparation and then placed on the media and incubated at 35°C for 18-24 hours. For negative control used blank for antibacterial This experiment did three repetitions as a comparison or control is also done isolate concentration test using chloramphenicol 30mcg/disc as a positive control. Growth inhibition against bacteria is seen from the presence or absence of an inhibition zone formed. The inhibition zone formed in the form of a clear area (clear zone) was measured with a vernier scale.

2.6.2. Minimum Inhibitory Concentartion (MIC) and Minimum Bactericidal Concentration (MBC) of C. citratus and C. nardus essential oil

In tube 1 put 0.5 ml of 40% lemongrass oil and add 0.5 ml of sterile broth then vortex until homogeneous, from tube 1 pipette 0.5ml put in tube 2 and add 0.5 ml of sterile broth in vortex, from tube 2 pipette 0.5 ml put in tube 3 then add 0.5 ml of sterile broth vortex until homogeneous, from tube 3 pipette 0.5 ml then add 0.5 ml of sterile broth vortex homogeneous then discard 0.5 ml. Put 0.1 ml of 25%T bacterial suspension in each of the four tubes, then vortex ad homogeneous and incubated for 18-24 hours. Then pipette 0.1 ml from each tube

and put it on each solid agar and incubate for 18-24 hours and observe whether there is growth on each petri dish. BHI and BHIA media were used for *Propionibacterium acnes* bacteria while NA and NB media were used for *Staphylococcus epidermidis* bacteria.

2.7. Microemulsion formulation

Microemulsions were prepared by mixing 20 ml of essential oil with a concentration of 40% which had been diluted by tween 80 with glyceryl caprylate, PEG 40 Hydrogenated Castor oil, and capric triglyceride in various compositions. The composition of ingredients that can produce microemulsions in the fastest time was formulated into skincare preparations consisting of 50% (v/v) microemulsion, 0.1% sodium benzoate, 0.1% sodium metabisulfite, 0.1% Na EDTA, and up to 100% water. The mixture was shaken in a bottle to form a clear and homogeneous microemulsion.

2.8. Microemulsion physical evaluation results

Microemulsion preparations were observed including color, odor, homogeneous or broken microemulsion and clarity. Microemulsion type testing is done by dilution method. This test is carried out by dissolving the sample into the water phase (1:100) and the oil phase (1:100). If the sample dissolves completely in distilled water, the microemulsion type belongs to the oil-in-water (O/W) type, while if the sample dissolves completely in the oil phase, the microemulsion type belongs to the water-in-oil (W/O) type. Droplet size was measured using a particle size analyzer with Malvern instrument. Zeta potential of droplets was measured using Zetasizer. The morphology of the preparation was measured using Transmission Electron Microscopy (TEM).

2.9. Evaluation of skin care formulations containing microemulsions of essential oils

The parameters of color testing was carried out organoleptically. The density test was carried out using a pycnometer. The pH measurement performed using a pH meter. Stability test was carried out using centrifugation test at 3750 rpm for five times 60 minutes and 10,000 rpm for 30 minutes which is equivalent to the effect of gravity for one year.

2.10. Irritation test

The test animals used were male albino rabbits weighing ± 2 kg 8-12 weeks age from IPB University . At least 24 hours before testing, the animal's fur must be shaved on the back area of approximately 10 x 15 cm. Shaving starts from the shoulder blade area (shoulder) to the groin bone (waist bone) and half down the body on each side. A total of 0.5 mL of essential oil microemulsion preparation. essential oil nanoemulsion preparation was applied first on gauze and then attached to the skin and covered with a non-irritant plaster. 3 test animals were used to observe the presence or absence of erythema and edema, response assessment was carried out at hours 1, 24, 48, and 72.

3. RESULTS

3.1. Plant determination

The findings of plant identifications conducted at the Herbarium Bogoriense, a research facility affiliated with the Indonesian Institute of Sciences' Research Center for Biology, revealed that the utilized plant species were *Cymbopogon nardus* (L.) and *Cymbopogon citratus* (DC.) Stapf, both belonging to the Poaceae family.

3.2. *C. citratus* and *C. nardus* essential oil quality parameters

The yield of the steam distillation of *C. nardus* was 0.67% and *C. citratus* was 0.4% against fresh samples. Table 1 displays the quality parameter of the essential oil derived from *C. citratus* and *C. nardus*, as examined in this study. The obtained results adhere to the standards set by ISO 3217:1974(en) for *C. citratus* essential oil and ISO 3849:2003(E) for *C. nardus* essential oil. This implies that the resultant product possesses the potential to serve as a primary component for microemulsion formulation.

Table 1. Quality parameters of *C. citratus* and *C. nardus* essential oil

Parameters	*C. citratus* essential oil		*C. nardus* essential oil	
	Specification*	Results	Specification**	Results
Appearance	Clear, mobile liquid	According to the standard	Clear, sometimes slightly opalescent, mobile liquid	According to the standard
Colour	Pale yellow to orange-yellow.	According to the standard	Pale yellow to pale brownish yellow	According to the standard
Fragrant	Characteristic with a strong note of citral.	According to the standard	Leafy, earthy	According to the standard
Density (20 °C)	0.872 – 0.897	0.895 ± 0.001	0.891 – 0.910	0.899 ± 0.001
Refractive index (20 °C)	1.483 – 1.489	1.486 ± 0.002	1.479 – 1.490	1,483 ± 0.003
Optical Rotation (20 °C)	-3 ° to +1 °	- 0.8 ± 0.1 °	-25 ° to -12 °	-14 ± 0.1 °
Solubility	Soluble in 70% ethanol	Soluble at 1:10	1:2 (ethanol 80%)	1:2 (ethanol 80%)

Data was given in mean+SD, n=3, * ISO 3217:1974(en); ** ISO 3849:2003(E)

3.3. Chemical Composition of *C. citratus* and *C. nardus* essential oil compounds with GC-MS

Results of the analysis of chemical compounds using gas chromatography-mass spectrometry (GC-MS) are presented in Table 2 and Table 3. These tables display the composition of 14 components found in *C. nardus* essential oil and 10 components found in *C. citratus* essential oil, both of which contain various antimicrobial agents. The primary constituents of the essential oil derived from *C. citratus* were found to be alpha-citral (37.8%) and beta-citral (27.91%), as depicted in Figure 1. On the other hand, the predominant components of the essential oil obtained from *C. nardus* were citronellal (48.6%) and trans-geraniol (25.77%), as illustrated in Figure 2.

Table 2. Examination of chemical compounds result from *C. citratus* essential oil

Retention Time	Quality	Compounds	Content (%)
6.368	97	Beta-myrcene	3.34
11.963	96	5-Hepten-2-one,6-methyl	2.86
18.982	97	Citronellal	1.99
22.459	95	Beta-Linalool	1.38
29.287	97	Beta-Citral	27.91
32.025	97	Alpha-Citral	37.80
33.949	98	Citronellol	3.94
34.635	99	Delta-Cadinene	1.84
38.006	95	Trans-Geraniol	7.17
49.287	87	(+)-1(10)-Aristolene	1.83
49.649	94	Octadecanoic acid	1.87
50.226	42	Beta-Bisabolene	1.26
52.278	99	Oleic acid	1.07
52.383	99	(9E)-9-octadecenoic acid	1.97

Table 3. Examination of chemical compounds result from *C. nardus* essential oil

Retention Time	Quality	Compounds	Content (%)
6.735	99	Alpha-Limonene	4.24
18.363	98	Citronellal	48.60
21.992	96	Linalol	1.05
32.259	97	Alpha-Amophene	2.43
32.859	91	Geraniol Acetate	2.49
33.497	98	Citronellol	5.60
37.702	96	Trans-Geraniol	25.77
47.664	91	Elenol	1.85
51.549	98	Cis-Isoeugenol	1.15
54.773	99	Palmitic Acid	3.95

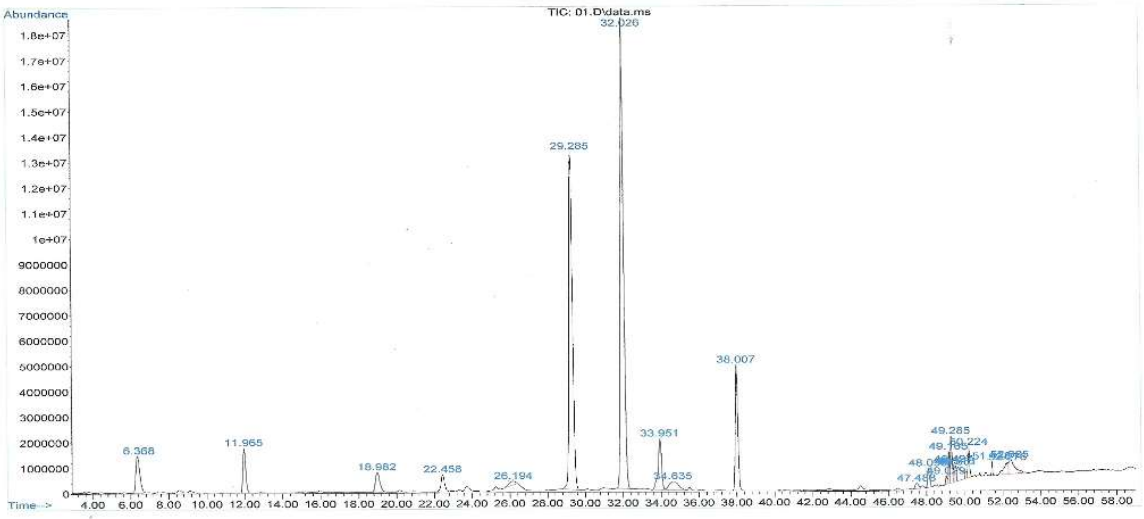

Figure 1. Chromatogram of GC-MS test results for *C. citratus* essential oil

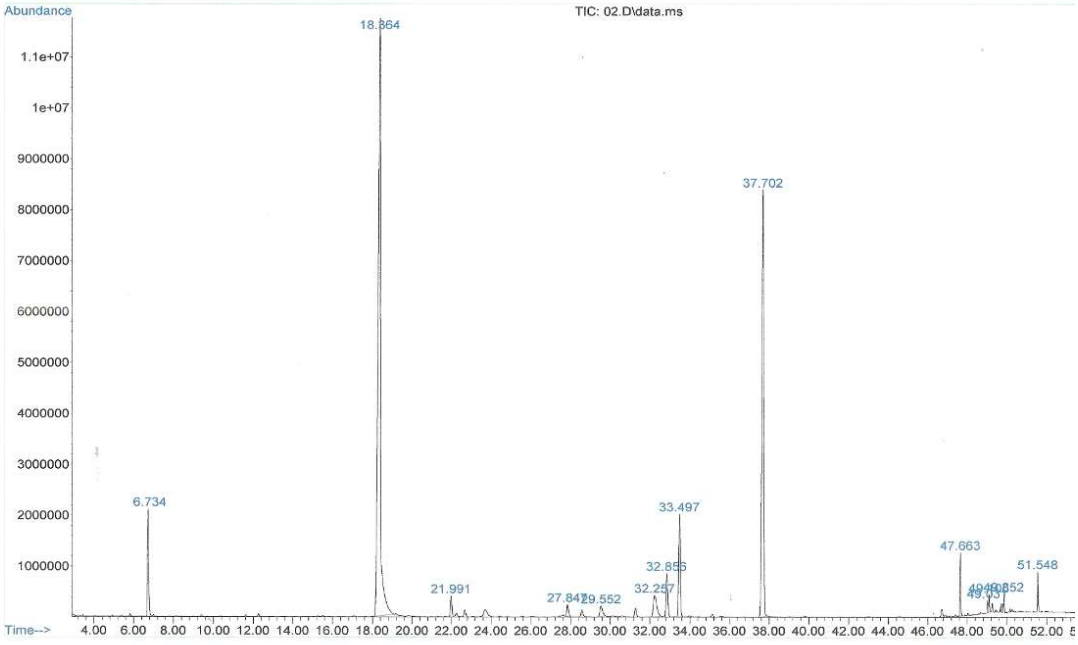

Figure 2. Chromatogram of GC-MS test results for *C. nardus* essential oil

3.4. Zone of inhibition of *C. citratus* and *C. nardus* essential oil

Table 4 displays the observed zone of inhibition resulting from the application of essential oils derived from *C. citratus* and *C. nardus* in the scope of this particular study. The findings of the investigation indicate that the diameter of inhibition caused by *C. citratus* essential oil is significantly greater than that of *C. nardus* essential oil in relation to the inhibition of *P. acnes* and *S. epidermidis* ($p<0.05$).

3.5. Minimum Inhibitory Concentartion (MIC) and Minimum Bactericidal Concentration (MBC) of *C. citratus* and *C. nardus* essential oil

MIC and MBC of *C. citratus* and *C. nardus* essential oil in this study was shown in table 4. The MBC of *C. citratus* essential oil against *Propionibacterium acnes* and *Staphylococcus epidermidis* were found to be lower compared to *C. nardus* essential oil. The MBCs of essential oils derived from *C. nardus* and *C. citratus* against *P. acnes* were found to be 20% and 10%, respectively. Similarly, the MBCs of *C. nardus* and *C. citratus* essential oils against *S. epidermidis* were determined to be 40% and 10%, respectively. The MIC values of *C. nardus* essential oil were found to be approximately 10% to 20% when tested against both *P. acnes* and *S. epidermidis*.

The MIC values of the essential oil derived from C. citartus were found to be approximately 10% to 20% against *P. acnes* and 20% to 40% against *S. epidermidis*.

Table 4. Zone of inhibition, MIC, and MBC of *C. citratus* and *C. nardus* essential oil

Sample	Test microbes	Zone of inhibition (mm) (±SD)				Minimum inhibitory concentration			
		60%	40%	20%	Positive control	5%	10%	20%	40%
C. citratus essential oil	*P. acnes*	24.71 ± 1.1	19.98 ± 1.1	11.69 ± 1.2	25.3 ± 1.4	+	- (*)	-	-
	S. epidermidis	23.37 ± 1.2	18.65 ± 1.3	8.59 ± 0.9	1.42 ± 0.2	+	- (*)	-	-
C. nardus essential oil	*P. acnes*	10.78 ± 1.1	6.94 ± 0.9	3.36 ± 0.2	27.9 ± 1.5	+	+	- (*)	-
	S. epidermidis	12.85 ± 1.3	8.38 ± 0.9	4.57 ± 0.7	1.85 ± 0.2	+	+	+	(*)

P. acne: Propionibacterium acne; S. epidermidis: Staphylococcus epidermidis; SD: Standard deviation; (*) MBC

3.6. Microemulsion formation time

During the experiment, the microemulsion material contained in the bottle was thoroughly mixed until achieving a state of homogeneity. Subsequently, distilled water was introduced and mixed by shaking. The duration required for the formation of a microemulsion refers to the temporal interval necessary for the emergence of a visually transparent and self-assembled microemulsion. The experimental findings indicate that composition no. 3, consisting of *C. nardus* essential oil, and composition no. 6, containing *C. citratus* essential oil, exhibited the shortest duration for achieving the requisite microemulsion, as presented in Table 5. The formulation of microemulsion skin care treatments utilizes the composition that exhibits the shortest duration. The composition which produces the fastest time was used in the formulation of microemulsion skin care preparations.

3.7. Microemulsion physical evaluation results

Table 6 presents the outcomes of the physical assessment conducted on the two microemulsions. The microemulsion that was obtained exhibited characteristics of transparency, clarity, and homogeneity. The *Cymbopogon citratus* oil microemulsion (CCM) exhibits a yellow hue and emits a fragrance reminiscent of lemon, whereas the *Cymbopogon nardus* microemulsion (CNM) possesses a transparent white appearance and emanates a discernible perfume. Both COM and CNM were oil-in-water (o/w) emulsions prepared using the dilution method. The Figure 3 illustrates the spherical morphology of the globules generated in COM and CNM.

Table 5. microemulsion formation time of *C. citratus* and *C. nardus* essential oil

No	Microemulsion composition (%)					Time to form microemulsion (sec)	
	Essential oil : Tween 80 (40:60)	Glyceryl caprylate	Capric triglyceride	PEG 40 HCO	Aquadest	CCM	CNM
1	1	0.25	0.25	0.25	Ad to 100	9.96 ± 0.32	8.87 ± 1.12
2	1	0.5	0.25	0.25	Ad to 100	9.65 ± 1.31	7.07 ± 1.00
3	1	0.5	0.5	0.25	Ad to 100	5.43 ± 0.20*	5.73 ± 0.30
4	1	0.25	0.25	0.5	Ad to 100	7.71 ± 0.30	8.28 ± 0.90
5	1	0.5	0.25	0.5	Ad to 100	7.09 ± 1.20	7.16 ± 0.72
6	1	0.5	0.5	0.5	Ad to 100	7.09 ± 0.93	4.51 ± 0.52*

CCM*: C. citratus* oil microemulsion*;* CNM*: C. nardus* microemulsion; SD: Standard deviation; PEG-40 HCO: PEG-40 hydrogenated castor oil *fastest time

Table 6. Results of physical evaluation of the *C. citratus* oil microemulsion (CCM) and *C. nardus* microemulsion (CNM)

Parameters	CCM	CNM
Particle size (nm)	36.98 ± 1.45	27.83 ± 2.50
Polydispersity index	0.084 ± 0.020	0.127 ± 0.031
Zeta potential	-10,5	-13.8

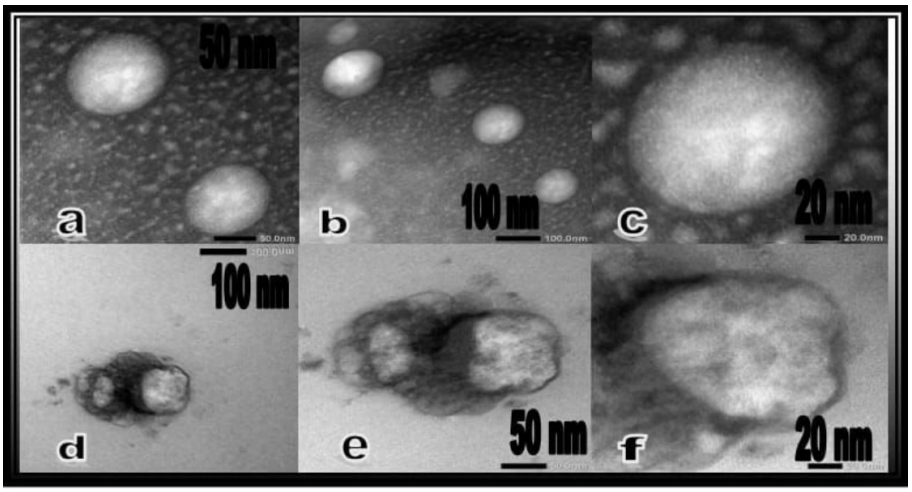

Figure 3. Morphological globule of *C. citratus* oil microemulsion (a, b, c) and *C. nardus* microemulsion (d, e, f)

3.8. Evaluation of skin care formulations containing microemulsions of essential oils

The results regarding the physical and chemical assessment of skin care products are presented in Table 7. The stability testing of two separate skincare microemulsions, one containing *C. citratus* and the other including *C. nardus*, was conducted using a centrifugator at varying times and speeds. The acquired results indicated that there was no phase separation observed in either of the microemulsions. The zone of inhibition of *C. citratus* and *C. nardus* essential oil in this study was shown in Table 8.

3.9. Irritation test results

The results of the skin tolerance test were shown in Table 9. No symptoms of irritation or skin edema were observed in rabbits following treatment with skincare products containing *Cymbopogon citratus* oil and *Cymbopogon nardus* oil. There was a notable absence of irritation and erythema in comparison to the control group. The rabbits exhibited a score of "0" for both edema and erythema during the whole observation period following the removal of the test item. The results indicate that the skin Primary Irritation Index score was 0.

Table 7. Results of physical and chemical evaluation of the *C. citratus* oil skincare (CCS) and *C. nardus* skincare (CNS)

Parameters	CCS	CNS
Color	Yellow transparant	White transparant
Homogeinity	Homogen	Homogen
pH	5.81 ± 0.02	6.32 ± 0.02
Density (g/mL)	0.986 ± 0.004	0.99 ± 0.006

Table 8. Zone of inhibition of The *C. citratus* oil skincare (CCS) and *C. nardus* skincare (CNS)

Test Sample	Zone of inhibition (mm) (±SD)	
	P.acnes	*S. epidermidis*
CCS	13.75 ± 1.45	8.41 ± 1.25
CNS	6.87 ± 0.80	5.24 ± 0.92
Positive control	29.13 ± 1.34	1.48 ± 0.67

COS: *C. citratus* oil skincare; CNS: *C. nardus* oil skincare; *P. acne: Propionibacterium acne*; *S. epidermidis: Staphylococcus epidermidis;* SD: Standard deviation

Table 9. Irritation test of The *C. citratus* oil skincare (CCS) and *C. nardus* skincare (CNS)

Sample test	Erythema	Udema
CCS	0	0
CNS	0	0
Blank	0	0
Control	0	0

COS: *C. citratus* oil skincare; CNS: *C. nardus* oil skincare

4. DISCUSSION

One of the bioactivities attributed to essential oils frequently employed in skincare is derived from *Cymbopogon nardus* L. and *Cymbopogon citratus* DC [10]. Essential oils that meet the requisite parameters can be employed in diverse applications, including but not limited to aromatherapy, insect repellents, pharmaceutical uses, food products, and cosmetics [11]. The ideal characteristic of essential oil offers numerous benefits and introduces potential alternative options that may be later utilized in various applications.

Upon the completion of the distillation process, it was seen that the extraction yield in this particular study was comparatively lower than the total oil yield reported by Singh for Citronella essential oil, which was documented as 0.79% [12]. The yield of essential oil was influenced by the procedures employed in the distillation process. The contemporary extraction apparatus exhibited superior distillation efficiency in extracting oil content compared to the conventional alcohol boiler, which demonstrated the lowest oil content.

The application of Gas Chromatography-Mass Spectrometry (GC-MS) The primary constituents found in the various tissues of cymbopogon species include Citronellal, Citronellol, Neral, Geraniol, Geranyl acetate, α-elemol, Geranial, and α-Eudesmol. *Cymbopogon citratus* DC and *Cymbopogon nardus* (L) Rendle exhibit potential antibacterial properties as alternatives to synthetic medications for combating microbial infections. The antibacterial powers of *Cymbopogon citratus* DC and *Cymbopogon nardus* (L) Rendle can be attributed to the presence of citronellal, citronellol, and other secondary metabolites. These compounds exhibit both synergistic and antagonistic effects. The precise mechanism responsible for the antifungal and antibacterial actions of EO remains uncertain. Nevertheless, there exists a hypothesis suggesting that the hydrophobic constituents have detrimental effects on the cytoplasmic membrane, either by impeding the process of sporulation or by initiating a series of events that lead to the leakage of cytoplasm, lysis of cells, and ultimately, demise [13].

The disc diffusion method was employed to evaluate the antibacterial properties of various oils and their primary ingredients against bacteria that are known to cause acne. Both oils demonstrated significant inhibitory zones against *Propionibacterium acnes* and *Staphylococcus epidermidis*, indicating potential antimicrobial activity. The essential oil derived from *C. citratus* exhibited the most extensive inhibitory clear zones, accompanied by the lowest minimum inhibitory concentrations (MICs) within the 10% range. The findings of this investigation are consistent with a previous study conducted by Bunrathep et al, in which it was observed that the primary component of lemongrass oil had the greatest effectiveness against both *P. acnes* and *S. epidermidis* [14]. Tween 80 serves as the primary surfactant, facilitating enhanced solubility of the oil phase. In this work, the oil phase surfactants employed were Glyceryl Caprylate and capric triglycerides, whereas PEG-40 hydrogenated castor oil was utilized as a cosurfactant. According to Djekic, the addition of an adequate quantity of a suitable surfactant leads to the complete solubilization of oil and water, resulting in the formation of single-phase systems known as Winsor IV microemulsions [15]. The observed duration in this investigation exhibited more efficiency compared to previous investigations that utilized tween 80 as the primary surfactant [16]. The variability in the production period of microemulsions can be attributed to a multitude of causes. The utilization of a surfactant possessing an optimal hydrophilic-lipophilic balance (HLB) is a crucial determinant in the establishment of stable emulsions, as highlighted by Peng et. al. [17]. The relevant parameters in this study were the water to oil ratio, stirring speed, and stirring time [18].

The size of the globules obtained is in accordance with the specifications for microemulsions. According to a study conducted by Formariz et al. (2010), the particle size of microemulsions typically falls between the range of 10 to 250 nm [19]. The droplet size of an emulsion is influenced by several factors, including the composition of the dispersed phase, interfacial characteristics, viscosity of the phases, hydrophobic emulsifier with a low hydrophilic-lipophilic balance (HLB) and a greater affinity to the organic phase, shear rate applied during emulsification, and the solubility of the oil phase in water. According to Peng et al. (2010) and Tabibiazar & Hamishehkar (2015), it has been observed that the potential cause for the increase in droplet size, resulting from an increase in dispersion phase concentration, can be attributed to the heightened contact and subsequent coalescence of larger droplets [17,20,21]. Furthermore, in instances where the concentration of the dispersed phase is elevated, it is possible that the concentration of surfactant may be insufficient to adequately coat all freshly generated droplets.

The Particle Size Distribution Index (PDI) is a metric used to assess the degree of uniformity in the distribution of particle sizes within a given sample. Hence, the findings of this study indicate that both microemulsions exhibited homogeneity. Both microemulsions exhibited a negative charge, which can be

attributed to the presence of unbound fatty acids [22]. The findings from the zone of inhibition analysis indicated that microemulsion skincare formulations containing *Cymbopogon citratus* exhibited notably greater inhibitory effects compared to *Cymbopogon nardus* ($p<0.05$) against the bacteria *Propionibacterium acnes* and *Staphylococcus epidermidis*.

Understanding the possibility for skin sensitization is a crucial requirement for conducting comprehensive assessments of both hazard and risk. Accurate skin sensitization data for each specific chemical is crucial, particularly in cases where direct contact with the skin is anticipated. The dermal irritation test is a method used to assess the potential irritancy of a substance when it comes into contact with the skin. The toxicity screening of the *Cymbopogon citratus* oil microemulsion skincare (CCS) and *Cymbopogon nardus* microemulsion skincare (CNS) formulation is crucial for conducting a comprehensive assessment of the potential risks posed to the skin. This screening process involves the fundamental characterisation of the afore mentioned skincare formulations. The skin irritating impact of the created microemulsion was assessed, and it was found that there were no observable signs of erythema or edema when the PII value was equal to 0. This suggests that the herbal formulation prepared is suitable for safe application on the skin. The findings of this study indicate that lemongrass oil possesses potential as a substitute for citral in pharmaceutical formulations due to its comparable antifungal properties, cost-effectiveness, and low toxicity [23].

5. CONCLUSION

The essential oils derived from *C. citratus* and *C. nardus* exhibit antimicrobial activity against the microorganisms associated with Acne vulgaris. The microemulsion that was obtained both the physical and chemical criteria. Hence, there was potential for the further advancement and commercial application of this treatment for acne.

Acknowledgements: Authors would like to thank to Universitas Pancasila.

Author contributions: All the authors have contributed equally.

Conflict of interest statement: The author declares no conflict of interest

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